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## Root biomechanical properties during establishment of woody perennials

Boldrin, D.; Leung, A. K.; Bengough, A. G.

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1                   Root biomechanical properties during establishment of woody perennials

2                   Boldrin, D. <sup>a, b</sup>, Leung, A. K. <sup>a</sup>, and A. G. Bengough <sup>a, b</sup>

3  
4                   <sup>a</sup> *School of Science and Engineering, University of Dundee, Dundee, DD1 4HN, United*  
5                   *Kingdom*

6                   <sup>b</sup> *The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, United Kingdom*

7   **Corresponding author** Anthony K. Leung

8   E-mail: [a.leung@dundee.ac.uk](mailto:a.leung@dundee.ac.uk)

9   Tel: 01382384390

10   **ABSTRACT**

11   *Background and aims* Soil bio-engineering using vegetation is an environmentally friendly  
12   solution to stabilise soil slopes. This study investigates tensile strength, Young's modulus,  
13   and root diameter relationships for establishing woody perennials.

14   *Methods* Specimens of ten woody European shrubs and small trees were transplanted into  
15   sandy loam soil to establish for six months. Root tensile strength and Young's modulus were  
16   measured as well as the root length-diameter distribution. The effect of root water status on  
17   root diameter was evaluated for Scotch Broom.

18   *Results* More than half of the root length for all species was thinner than 0.5 mm diameter.  
19   Typical tensile strengths were <40 MPa, with Young's modulus <600 MPa. Negative power  
20   relationships between root strength and root diameter existed only for Gorse and Spindle,  
21   whilst Blackthorn, European Box and Holly showed slight increase in tensile strength with  
22   diameter. Hawthorn, Hazel and Privet showed rapid initial increase in strength with diameter  
23   followed by strength decrease with diameter, post-peak. Young's modulus was linearly  
24   related to tensile strength for all ten species ( $P < 0.001$ ;  $R^2$  values 17% to 64%). Root  
25   diameter, investigated for Scotch Broom, depended strongly on root water potential and root  
26   water content by mass). Root water content could influence considerably the calculations of  
27   tensile strength.

28   *Discussion and conclusion* Root strength-diameter relationships often do not follow a  
29   negative power law, and depends strongly on taxa. Young's modulus was strongly related to  
30   tensile strength of roots for certain species. Water status of roots strongly influences root  
31   diameter and hence strength and Young's modulus properties, and must be controlled  
32   carefully in experiments.

## 1. Introduction

In recent years, soil bio- and eco-engineering using plants has been recognised as an environmental-friendly and low CO<sub>2</sub>-emission solution for soil stabilisation, as compared to existing traditional “hard” engineering solutions such as soil nailing and piling (Inui et al. 2011; Stokes et al. 2008; Stokes et al. 2014). Plant roots can increase soil shear strength through mechanical and hydrological reinforcement (Pollen-Bankhead and Simon 2010; Leung and Ng 2013; Saifuddin and Osman 2014; Veylon et al. 2015; Leung et al. 2015). Whilst soil is generally weak in tension and strong in compression, roots are strong in tension. Root-permeated soil thus represents a type of composite material with enhanced mechanical properties beneficial for slope stabilisation (Simon and Collison 2002; Fan and Su 2008; Mickovski et al. 2009).

Root mechanical reinforcement depends on root system morphology, root number, diameter, tensile strength and stiffness (Young’s modulus; the initial linear part of a tensile stress-strain curve) (Mickovski et al. 2007; Mickovski et al. 2009; Stokes et al. 2009; Loades et al. 2010; Osman and Barakbah 2011; Ghestem et al. 2014b; Saifuddin et al. 2015). Root length is one of the most studied traits, which is often correlated with plant growth rate and plant’s ability to stabilise soil in disturbed areas (Stokes et al. 2009). The number and the relative amount of fine and coarse roots play a major role in soil stabilisation. While coarse roots (diameter > 10 mm) may act as a structural element like a soil nail, fine roots (diameter < 2 mm) permeated in the soil can create a membrane-like structure to protect soil from surface erosion (Stokes et al. 2009). Ghestem et al. (2014b) found that (i) the total length of coarse roots above a shear plane and (ii) the fine root density below the plane govern the contribution of root reinforcement to the increase in soil shear strength.

Many studies on root mechanical reinforcement have assessed the relationship between root diameter and root tensile strength (Mattia et al. 2005; Bischetti et al. 2009; Preti and Giadrossich 2009; Mickovski et al. 2009; Ghestem et al. 2014a). A negative power law has been commonly used to describe the variation of root tensile strength with root diameter for several plant species (Mao et al. 2012).

$$T_r = \alpha d^\beta \quad (1)$$

where  $\alpha$  and  $\beta$  are empirical coefficients that are species-specific.  $\beta$  is always less than zero, so roots with larger diameters would have a lower tensile strength. Mao et al. (2012) listed  $\alpha$  and  $\beta$  for 81 species of grass, forbs, shrubs and trees reported in the literature. Eqn (1) has

69 been commonly used as a predictive model to estimate the mechanical reinforcement that can  
70 be provided by roots through the so-called root cohesion (Mao et al. 2012). This general  
71 negative power law has also been sometimes used in the design of appropriate root analogues  
72 for bio-engineering research (Liang et al. 2014; Liang et al. 2015; Meijer et al. 2016).

73 The common negative power law fitting, however, is often able to explain only a  
74 small fraction of the variability in tensile strength-diameter relationship (Mattia et al. 2005;  
75 Ghestem et al. 2014a; Vergani et al. 2014). Root biomechanical properties change over time  
76 as a function of root chemical composition (i.e., cellulose and lignin content) (Genet et al.  
77 2005; Saifuddin and Osman 2014; Zhang et al. 2014), root type (Loades et al. 2013), root age  
78 (Dumlao et al. 2015; Loades et al. 2015), root decay (Watson et al. 1999), moisture content  
79 (Yang et al. 2016) and in response to mechanical stress (Chiatante et al. 2003; Loades et al.  
80 2013). Although the tensile strength-diameter relationship has been generally considered to  
81 follow a negative power law model, the physical basis of such relationships is still not clear  
82 nor its optimal use for different species or conditions. In particular, there is a lack of  
83 information and understanding of root biomechanical properties during early stages of plant  
84 establishment (i.e., first year since transplanting or planting), which is the most critical period  
85 for slope stabilization by soil bio-engineering. Live plant material often needs months or even  
86 years to develop sufficient strength to stabilize soils (Osman and Barakbah 2011; Stokes et al.  
87 2014; Sidle and Bogaard 2016). Based on previous field and modelling studies, a forest re-  
88 establishment period of approximately 3 to 20 years from forest harvesting is needed to  
89 recover the pre-harvest conditions of root strength and slope stability (Sidle and Bogaard  
90 2016). This period represents a “temporal window” that coincides with an increase in  
91 landslide rate of about 2 to 10-fold compared to undisturbed forests. Indeed, a study on the  
92 re-establishment of pioneer vegetation (Schmidt et al. 2001) reveals that during the first 7  
93 years since forest harvesting, root cohesion (i.e., the additional strength gain by soil due to  
94 roots) remained less than 3 kPa. The root cohesion for coniferous and hardwood vegetation  
95 was recovered to values higher than 10 kPa after almost a decade. Moreover, Preti and  
96 Giadrossich (2009) highlighted reduced root growth to depth for transplanted plants when  
97 compared with naturally regenerated plants of the same age in the same area.

98 The experiments reported in this paper aim to evaluate the biomechanical properties  
99 of ten selected shrubs and small trees widespread in Europe. The objectives are (i) to measure  
100 and quantify root biomechanical properties during their early stage establishment, which  
101 represents a particularly challenging period for these plants; (ii) analyse and compare the  
102 tensile strength-diameter and Young’s modulus-diameter relationships of the ten woody

species. Preliminary experiments were also performed on a single species to evaluate the effects of root water status and related diameter change on root tensile strength estimation, as significant changes in root diameter can occur with change in plant water status (Huck et al., 1970; Carminati et al, 2009, 2013).

## 2. Material and methods

### 2.1. Selected plant species

Ten woody species, which would grow into shrubs or small trees, were selected for testing in this study. These include *Buxus sempervirens* L., *Corylus avellana* L., *Crataegus monogyna* Jacq., *Cytisus scoparius* (L.) Link, *Euonymus europaeus* L., *Ilex aquifolium* L., *Ligustrum vulgare* L., *Prunus spinosa* L., *Salix viminalis* L. and *Ulex europaeus* L. Their family, common name, height range, age and the acronym used throughout this study are summarised in Table 1. These species have been suggested as suitable plants for soil eco- and bio-engineering applications (Marriott et al. 2001; Coppin and Richards 2007; Norris et al. 2008; Beikircher et al. 2010) and are suited to a Nord European wet maritime climate. These species have been previously tested for soil hydrologic reinforcement (Boldrin et al. 2016; Boldrin et al. 2017). Plants that were 30 – 80 cm tall and older than one year were selected for testing in this study. This plant size range is considered representative of that commonly adopted for soil bio- or eco-engineering projects (see online document 1).

Eight replicates of bare root plants per species were transplanted in pots (0.24 m in diameter; 0.009 m<sup>3</sup> in volume) with arable soil during the dormant season. Following transplanting, plants were kept in a glasshouse, where no additional light or heating was provided. The temperature of glasshouse was thus close to the outdoor temperature during the entire experiment. The soil used in this study was collected from Bullionfield, The James Hutton Institute, Dundee, UK. It was a sandy loam, which comprised of 71% sand, 19% silt and 10% clay contents (Loades et al. 2013). The soil (sieved < 10 mm; water content 0.15 g/g) was dynamically compacted in five layers in pots to obtain an initial dry density of 1200 kg m<sup>-3</sup>. The soil packed at this density had a water content at field capacity (5 kPa suction) equal to 0.25 g g<sup>-1</sup> and 0.08 g g<sup>-1</sup> at the permanent wilting point (1500 kPa suction). After soil compaction and transplantation, each planted pot was irrigated depending on the season and glasshouse temperature. No fertilizer was added to all planted pots.

**Table 1**

A list of the ten species selected for testing in this study. Their family, common name, height, age and the acronym used throughout this study are reported. \* indicate the propagation by cutting. All plants were supplied by British Hardwood Tree Nursery, Gainsborough, UK.

| <i>Species</i>                     | <i>Family</i> | <i>Common name</i> | <b>Height, cm</b> | <b>Age, year</b> | <i>Acronym</i> |
|------------------------------------|---------------|--------------------|-------------------|------------------|----------------|
| <i>Buxus sempervirens</i> L.       | Boxaceae      | European Box       | 30-40             | 3                | Bs             |
| <i>Corylus avellana</i> L.         | Betulaceae    | Hazel              | 60-80             | 2                | Ca             |
| <i>Crataegus monogyna</i> Jacq.    | Rosaceae      | Hawthorn           | 60-80             | 2                | Cm             |
| <i>Cytisus scoparius</i> (L.) Link | Fabaceae      | Scotch broom       | 40-60             | 2                | Cs             |
| <i>Euonymus europaeus</i> L.       | Celastraceae  | Spindle            | 60-80             | 2                | Ee             |
| <i>Ilex aquifolium</i> L.          | Aquifoliaceae | Holly              | 40-60             | 2                | Ia             |
| <i>Ligustrum vulgare</i> L.        | Oleaceae      | Privet             | 60-80             | 2                | Lv             |
| <i>Prunus spinosa</i> L.           | Rosaceae      | Blackthorn         | 60-80             | 1                | Ps             |
| <i>Salix viminalis</i> * L.        | Salicaceae    | Willow             | 60-80             | 1                | Sv             |
| <i>Ulex europaeus</i> L.           | Fabaceae      | Gorse              | 40-60             | 2                | Ue             |

## 2.2. Measurements of root length per diameter classes

After four months establishment, roots of five replicates per species were washed free from soil using a set of sieves from 2 to 0.5 mm and stored in ethanol (70%) at 5°C. Representative subsamples of the root system (10% of root system by weight) were scanned using WinRhizo (Regent Instruments Inc.) to determine root lengths per diameter classes (0.1 mm interval width). The measured length and dry mass of these root subsamples were used to obtain the specific root length (SRL; root length per unit mass). The entire root system of each species was oven-dried at 60 °C to determine the dry root biomass. The total length per each diameter class was then estimated by multiplying the dry root biomass by the SRL and the percentage of each diameter class. Thick roots (>5 mm diameter), if present, were processed and analysed separately to avoid errors in estimating root length.

## 2.3. Measurements of root biomechanical properties

Six months after transplanting, three replicates of planted pots per species were used to measure the root biomechanical properties, including tensile strength and Young's modulus. Root systems were washed free from soil using the identical procedures described above. Then, all root samples were stored at 5 °C in sealed plastic bags with moist blotting paper and subsequently tested for their bio-mechanical properties within three days after root washing (Loades et al. 2013).

Root segments of 100 mm were selected for testing from the root systems. Root diameter was measured at three points along each root segment using a stereo microscope and graticule (Leica, Milton Keynes, United Kingdom). Mean root diameter was then calculated for each root segment. Tensile tests of individual root segments were performed using a universal testing frame (Instron 5966, Norwood, MA, USA) at an extension rate of 2 mm min<sup>-1</sup>. Tensile tests were carried out using 50 N and 500 N load cells, which were chosen according to the range of root diameter being tested. To avoid slippage of roots in the clamps, both manually-tightened and pneumatic clamps were used, depending on the root diameter. Pneumatic clamps with a pressure ranging from 100 to 250 kPa were used for roots with diameter larger than 2 mm. The tensile strength and Young's modulus of each root section were determined from the corresponding stress-strain curve (Loades et al. 2013). Tensile strength ( $T_r$ ) was obtained using Eqn (2),

$$T_r = \frac{F}{\pi \left( \frac{d^2}{4} \right)} \quad (2)$$

where  $F$  is the peak force required to break a root and  $d$  is the root diameter. Young's modulus ( $E$ ) was calculated for the initial linear part of a tensile stress-strain curve:

$$E = \frac{FL_o}{\pi \left( \frac{d^2}{4} \right) \Delta L} \quad (3)$$

where  $F$  is the applied force;  $L_o$  is the initial length of the root sample before testing; and  $\Delta L$  is the change in root length (De Baets et al. 2008; Loades et al. 2013). Root diameter is therefore a key parameter that could affect both the values of root tensile strength and Young's modulus (Eqns 2 & 3).

#### 2.4. Evaluation of the effects of root water status on root diameter

An initial experiment was performed to evaluate the relationship between root drying (gravimetric water content) and root diameter. Roots of one of the ten woody species, *C. scoparius*, which is one of the most common woody species on Scotland slopes subjected to disturbance, were selected for testing. Indeed, this species would naturally colonize man-made slopes/embankments during primary succession. Immediately after the collection of root samples, they were submerged in distilled water for 48 h. Then, three root sections (60 mm length and 4.3 – 5.1 mm diameter) were weighed on an electronic 4-decimal-place balance (ExplorerPro, Ohaus, Switzerland) and then left to dehydrate on a bench. During

dehydration, their weights were measured 14 times at frequent intervals during four days. The diameters of root sections were measured immediately after each weight measurement. Finally, all the samples were oven-dried at 60 °C for 72 h until a constant weight was resulted.

In a second experiment, to evaluate the effect of root water potential on root diameter, eight root sections (20-30 mm length and 2.5 – 4 mm diameter) were placed in sampling holders (diameter 40 mm; height 10 mm) to measure their water potential (MPa) using a dew-point hygrometer (WP4-T, Decagon Devices). Each root sample was then left to dehydrate. The root water potential and root diameter were measured 22 times at frequent intervals during five days, during progressive dehydration of the root material.

## 2.6. Statistical analysis

Statistical analysis was performed using GenStat 17<sup>th</sup> Edition (VSN International) and SigmaPlot13 (Systat Software Inc). Significant differences were assessed with one way-ANOVA, followed by post hoc Tukey's test. Results were considered statistically significant when P-value  $\leq 0.05$ .

## 3. Results

### 3.1. Root length per diameter class

Fig. 1 shows the root length percentage per diameter class recorded in five replications per species. *S. viminialis* (Sv) has the largest percentage of very fine roots (< 0.1 mm). Indeed, the percentage of the total length of very fine roots in *S. viminialis* exceeded 40%, but it did not reach 20% in other species. In most of the tested species, 40 to 90% of the total root length measured was for the root diameter classes between 0.1 and 0.5 mm. In all tested species, roots with diameters larger than 1 mm constituted less than 10% of the total root length.

### 3.2. Root tensile strength

Root tensile strength was determined for at least 45 root segments for each species, varying between 0.2 and 5.8 mm in diameter (Table 2). Boxplots of root tensile strength per each tested species are showed in Fig. 2A. The average tensile strength of species varied between 7.1±0.9 MPa (*B. sempervirens*) and 23.2±1.2 MPa (*C. monogyna*). Moreover, *C. monogyna* has the highest root tensile strength per individual root section (41.8 MPa), which was recorded in a root segment with 0.75 mm diameter.



The measured root tensile strength-diameter relationships of ten species highlighted three different trends, which may be described by three different types of fitting equations (Fig. 1; Table 2). We observed a negative power trend only in *E. europaeus* and *U. europaeus*, while *B. sempervirens*, *I. aquifolium* and *P. spinosa* showed an increase in tensile strength with diameter. Moreover, *C. avellana*, *C. monogyna* and *L. vulgare* seem to show an initial increase in strength with diameter, but beyond the peak strength, a significant strength reduction with diameter followed. These bimodal trends may be described by a critical exponential equation (Table 2). It should be noted that the range of root diameters where the peak tensile strength is found for both *C. avellana* and *L. vulgare* was consistently between 1.5 and 2.5 mm. Although the value of  $R^2$  of all three models indicate that only 17% to 36% of variation is typically accounted, our data showed that the strength-diameter relationship does not always follow a negative power law in several species. *C. scoparius* and *S. viminalis* did not show any significant relationship between root tensile strength and root diameter.

**Table 2**

Summary of the data of root tensile strength and Young's modulus (Mean  $\pm$  standard error of mean) per each tested species. Best-fit equation, P-values and  $R^2$  are given for the strength and Young's modulus-diameter relations. n.s. indicates the lack of significant relation between the two variables.

| Species                | Diameter range, mm | n samples | Average tensile strength, MPa | Fitting equation - Tensile strength<br>[P-value; Adj. $R^2$ ]          | Average Young's modulus, MPa | Fitting equation - Young's modulus<br>[P-value; Adj. $R^2$ ]         |
|------------------------|--------------------|-----------|-------------------------------|--|------------------------------|--|
| <i>B. sempervirens</i> | 0.7-2.3            | 46        | 7.1 $\pm$ 0.9                 | $f = -8.42 + 13.72 \cdot x$<br>[<0.001; 0.35]                          | 211.8 $\pm$ 21.7             | $f = -144.40 + 315.36 \cdot x$<br>[<0.001; 0.35]                     |
| <i>C. avellana</i>     | 0.4-5.6            | 54        | 15.3 $\pm$ 0.7                | $f = -1.9 + (2.9 + 24.9 \cdot x) \cdot (0.624^x)$<br>[<0.001; 0.29]    | 246.7 $\pm$ 10.9             | $f = -3112 + (3345 + 375 \cdot x) \cdot (0.909^x)$<br>[<0.001; 0.28] |
| <i>C. monogyna</i>     | 0.4-5.1            | 49        | 23.2 $\pm$ 1.2                | $f = 8.95 + (-12.7 + 64.2 \cdot x) \cdot (0.349^x)$<br>[<0.001; 0.24]  | 242.3 $\pm$ 16.9             | $f = 248.63 \cdot x^{-0.30}$<br>[0.003; 0.16]                        |
| <i>C. scoparius</i>    | 0.5-3.5            | 54        | 14.2 $\pm$ 0.8                | n.s.   | 203.1 $\pm$ 11.3             | n.s.   |
| <i>E. europaeus</i>    | 0.4-4.0            | 61        | 14.5 $\pm$ 0.9                | $f = 15.19 \cdot x^{-0.46}$<br>[<0.001; 0.18]                          | 221.4 $\pm$ 14.8             | $f = 232.11 \cdot x^{-0.49}$<br>[<0.001; 0.18]                       |
| <i>I. aquifolium</i>   | 0.5-2.9            | 49        | 7.2 $\pm$ 0.5                 | $f = 3.66 + 3.66 \cdot x$<br>[0.002; 0.17]                             | 157.3 $\pm$ 8.3              | n.s.   |
| <i>L. vulgare</i>      | 0.8-5.8            | 74        | 14.7 $\pm$ 0.7                | $f = 10.41 + (-38.8 + 40.6 \cdot x) \cdot (0.451^x)$<br>[<0.001; 0.36] | 173.0 $\pm$ 7.3              | n.s.   |
| <i>P. spinosa</i>      | 0.2-4.9            | 61        | 9.5 $\pm$ 0.5                 | $f = 7.64 + 1.64 \cdot x$<br>[<0.001; 0.19]                            | 150.9 $\pm$ 8.2              | $f = 109.75 + 35.31 \cdot x$<br>[<0.001; 0.28]                       |
| <i>S. viminalis</i>    | 0.4-5.5            | 45        | 10.9 $\pm$ 0.6                | n.s.   | 148.9 $\pm$ 11.5             | n.s.   |
| <i>U. europaeus</i>    | 0.4-2.7            | 53        | 17.2 $\pm$ 0.9                | $f = 16.61 \cdot x^{-0.46}$  | 323.2 $\pm$ 22.9             | n.s.   |

|  |  |  |  |                |  |  |
|--|--|--|--|----------------|--|--|
|  |  |  |  | [<0.001; 0.25] |  |  |
|--|--|--|--|----------------|--|--|

### 3.3. Young's modulus of roots

Fig. 2B shows the boxplots of the Young's modulus of roots of each tested species. The average Young's modulus of species ranged between  $148.9 \pm 11.5$  MPa (*S. viminalis*) and  $323.2 \pm 22.9$  MPa (*U. europaeus*). Half of the tested species showed a relationship between the Young's modulus and root diameter (Fig. 1 and Table 2). Three different trends can be observed, as similarly found in the strength-diameter relationships. Young's modulus showed a significant correlation with root tensile strength in all species (Fig. 1 and Table 3). When considering all ten species data, the tensile strength could explain 37% of the overall variation in Young's modulus ( $R^2$ ). When the strength-Young's modulus relationship of each individual species was examined, the  $R^2$  was up to 80% (Table 3).

**Table 3**

Summary of P-value,  $R^2$  and equations coefficients ( $y_0$  and  $a \pm$  standard error) for the linear correlation between root tensile strength and Young's modulus ( $f = y_0 + a \cdot x$ ) in the tested species. "All species", *B. sempervirens*, *C. scoparius* and *L. vulgare* data were log transformed in the statistical analysis to calculate P-values and  $R^2$ .

| Species                | P-value | Adj. $R^2$ | $y_0$            | $a$            |
|------------------------|---------|------------|------------------|----------------|
| <i>B. sempervirens</i> | <0.001  | 0.84       | $66.5 \pm 15.1$  | $20.5 \pm 1.6$ |
| <i>C. avellana</i>     | <0.001  | 0.24       | $133.0 \pm 28.9$ | $7.4 \pm 1.8$  |
| <i>C. monogyna</i>     | <0.001  | 0.47       | $14.2 \pm 36.6$  | $9.9 \pm 1.5$  |
| <i>C. scoparius</i>    | <0.001  | 0.21       | $103.1 \pm 26.8$ | $7.0 \pm 1.7$  |
| <i>E. europaeas</i>    | <0.001  | 0.64       | $35.4 \pm 20.0$  | $12.8 \pm 1.2$ |
| <i>I. acqifolium</i>   | <0.001  | 0.45       | $75.5 \pm 14.2$  | $11.4 \pm 1.8$ |
| <i>L. vulgare</i>      | <0.001  | 0.46       | $89.9 \pm 16.0$  | $5.6 \pm 1.0$  |
| <i>P. spinosa</i>      | <0.001  | 0.44       | $35.5 \pm 17.9$  | $12.1 \pm 1.8$ |
| <i>S. viminalis</i>    | <0.001  | 0.37       | $27.7 \pm 25.2$  | $11.1 \pm 2.2$ |
| <i>U. europaeus</i>    | <0.001  | 0.28       | $97.7 \pm 53.3$  | $13.2 \pm 2.9$ |
| All species            | <0.001  | 0.37       | $83.7 \pm 8.4$   | $9.2 \pm 0.6$  |

### 3.4. Effects of root water status on root diameter

Roots subjected to dehydration showed a large decrease in diameter due to water loss (Fig 3A). Following dehydration, root diameter shrunk by 30% as compared with the initial diameter. The initial root water content ( $1.85 \pm 0.06$  g g<sup>-1</sup>) showed a large decrease of 67% in the first 24 h, which resulted in 19% reduction from the initial diameter, after which the reduction continued with a slower rate.

Fig 3B shows the sigmoidal relation between root diameter and root water potential. A significant increase in root shrinkage happened when root water potential was lower than -1 MPa and approached -1.5 MPa, which is the plant water potential that is conventionally recognized as the permanent wilting point of mesophytic plants (Kramer 1983).

## 4. Discussion

### 4.1. Root system morphology and its relation to mechanical reinforcement

The ten species tested showed substantial differences in root length per diameter class and root biomechanical properties (Figs 1 and 2), which influences their suitability for stabilising slopes as “ecological engineer plants” (Ghestem et al. 2014a).

In all ten species, more than half of the root length was thinner than 0.5 mm diameter, whilst <10% of root length was > 1 mm diameter. In particular, the largest percentage of very fine roots (<0.1 mm) was observed in *S. viminalis* (> 40%), while the percentage of very fine roots did not exceed 20% in the other nine species. Very fine roots can create a composite material with soil and hence increase its tensile strength better than coarse roots, which act more like soil nails. In fact, a large number of fine roots could limit surface erosion, decrease the number of cracks occurring on surface soil and stabilise the shallow soil more effectively than a small number of coarse roots, which can slip out of the soil upon soil mass sliding (Gyssels et al. 2005; Reubens et al. 2007; Stokes et al. 2009; Comino et al. 2010; Loades et al. 2010). Indeed, Diambra et al. (2013) have shown that soil reinforced by polypropylene fibres (with a diameter range from 0.03 to 0.1 mm) has a greater mechanical strength than the soil without fibres. The large percentage of very fine roots in *S. viminalis* resulted in a high specific root length (SRL; data reported in Boldrin et al. (2017)). In fact, the SRL of *S. viminalis* ( $64.5 \pm 9.0 \text{ m g}^{-1}$ ) was 2.5 times higher than the average value of the other nine species ( $24.8 \pm 2.2 \text{ m g}^{-1}$ ). SRL is linearly correlated with plant growth rate and root hydraulic conductivity (Eissenstat 1992). Plant growth rate is an important factor, when new man-made or natural slopes need to be stabilized by soil bio-engineering methods. Indeed, pioneer species with fast growth rate, such as *Salix sp.* and *Populus sp.*, are often preferred by soil bioengineers as they propagate quickly from cuttings (or “live poles”), permitting rapid stabilisation of unstable slopes (Wu et al. 2014). A large percentage of fine roots (i.e., large SRL), as observed in *S. viminalis* and *U. europaeus*, is thus a desirable trait for plants suitable to protect soil slopes against slipping and erosion (Stokes et al. 2009).

In addition to root length per diameter class and specific root length, root spatial distribution in the soil, is another morphological trait that could significantly affect the degree of root reinforcement. Indeed, the architecture of root systems and root depth in the soil dictate the effective reinforcement provided by roots to a potential shear plane (Fan and Chen 2010; Ghestem et al. 2014b). Monitoring root growth, and hence root spread, in the soil remains challenging, especially through non-destructive means (Oswald et al. 2008; Downie et al. 2012). Future work is needed in this area to better inform the effectiveness of mechanical reinforcement provided by soil bioengineering techniques.

#### 4.2. Root tensile strength of different species

The average root tensile strength differed significantly between species (Fig. 2A), with average values ranging between  $7.1 \pm 0.9$  MPa (*B. sempervirens*) and  $23.2 \pm 1.2$  MPa (*C. monogyna*). *C. monogyna* had the highest tensile strength per individual root segment (41.8 MPa), recorded in a root segment with 0.75 mm diameter. The values observed in this study were generally smaller than those reported in the literature. For instances, tensile strength values up to 731 MPa can be found in *Fagus sylvatica* (Bischetti et al. 2005). A list of mean tensile strengths for 67 species (root diameter = 0.5 – 15 mm) is reported in Stokes et al. (2008). Bischetti et al. (2005) found a maximum tensile strength of *C. avellana* (257 MPa) that was nine times greater than the maximum value recorded in this study (29 MPa). Similarly, for *S. viminalis*, we found a maximum tensile strength of 18 MPa, which is about 8 times smaller than that (150 MPa) reported in Mickovski et al. (2009). The differences between the values presented in this study and in the literature may be partially explained by the plasticity of root biomechanical properties in response to the growth environmental conditions, such as soil moisture and density (Loades et al. 2013). Indeed, plant adaptive changes (plasticity) enable roots to adjust to spatial and temporal heterogeneity, thus minimizing abiotic and biotic stresses (Stokes et al. 2009). Moreover, the transplanting and the consequent root turn-over (Watson 1987) in our study might have increased the percentage of younger roots, which are generally weaker than mature roots (Dumlao et al. 2015; Loades et al. 2015).

#### 4.3. Strength-diameter relationships

Our results have highlighted that the relationships between root tensile strength and root diameter do not necessarily follow the commonly-quoted negative power law model (Fig. 1).

In our study, the negative power law can be applied to only *E. europaeus* and *U. europaeus*. It should be emphasised that the negative power law regressions reported in the literature are often able to explain only a small portion of the variation ( $R^2$ ) in strength-diameter relationship (Mattia et al. 2005; Ghestem et al. 2014a; Vergani et al. 2014). Indeed, in a study carried out by Ghestem et al. (2014a), some  $R^2$  values for power law regressions of strength-diameter relationships of different species were as low as 0.10 (e.g., 0.04 for *Arthraxon hispidus* and 0.00 for *Ficus tikoua*).

*C. avellana*, *C. monogyna* and *L. vulgare* show a consistent rapid initial increase in strength with diameter but for thicker roots a significant weakening with increasing diameter followed. We hypothesize that these bimodal trends can be explained by the differences between root primary and secondary structures. In the primary structure, the cortex usually occupies the largest volume of most roots and consists mainly of highly vacuolated parenchyma cells with diffuse intercellular spaces (Gregory 2008). In general, cortex, which is a parenchymal tissue, is characterized by thin cell walls, lacking in cellulose and lignin, the two main structural components that contribute to tissue's strength (Niklas 1992; Genet et al. 2005; Zhang et al. 2014). Previous studies have shown that the strength of plant tissues is negatively correlated with porosity (i.e., intercellular space and lumens of vessel elements) and thin cell walls (Niklas 1992; Striker et al. 2007; Zhang et al. 2014). For our woody species, thin roots (<1 mm diameter) can be reasonably assumed to be young and less developed (i.e., primary structure and early stage secondary structure). These thin roots are hence expected to be weak in tensile strength.

In contrast, as the proportion of secondary xylem (thick lignified cell walls) increase and the cortex is lost, biomechanical performance of plant tissues would be improved. Indeed, Kokubo et al. (1989) reported a linear correlation between the proportion of volume occupied by cell walls and strength in barley stems. The development of secondary structure of root tissues may thus explain the peak strength for both *C. avellana* and *L. vulgare* for the diameter range of 1.5 – 2.5 mm.

In general, the root tensile strength at small root diameters (< 1 mm) has high variability, as reported in both the present study (Fig. 1) and in the literature (Mickovski et al. 2009; Ghestem et al. 2014a; Zhang et al. 2014). This may be partially explained by the transition between the late stage of primary structure and the early stage of secondary structure. During this transition, the cortex is isolated from the rest of the root. Hence cortex dies and it is sloughed off as a normal part of the ageing process (Gregory 2008). Therefore, primary and secondary structure roots may co-exist in the same diameter class, due to the two

contrasting processes: secondary xylem development (i.e., diameter increase) and cortex loss (i.e., diameter decrease). Despite having the same diameter, such roots have different tissue composition and hence biomechanical properties. In this case, the general use of the negative power law model for root tensile strength-diameter relation should be treated with caution. The model could substantially overestimate root mechanical reinforcement, especially in the range of very fine and fine roots that are the most represented in terms of root length in all tested species in this study (see Fig. 1).

The post-peak decrease in tensile strength with increasing root diameter is in agreement with most of the literature (Genet et al. 2005; Bischetti et al. 2009; Mickovski et al. 2009; Loades et al. 2013; Ghestem et al. 2014a). Zhang et al. (2014) explained the strength decrease with the decrease of lignin/cellulose ratio as diameter increases. However, in contrast with Zhang et al. (2014), Genet et al. (2005) explain the negative power law of strength-diameter relation with cellulose decrease as diameter increases, as observed in three conifer and two broadleaf species. Moreover, strength decrease can be explained by the increase in potentially weak points, from which fracture propagation may start, as root diameter increases. The decrease in tensile strength with diameter has also been reported for non-biological materials such as glass fibres (Griffith criterion) (Griffith 1921) and plastic root analogues (Liang et al. 2015; Meijer et al. 2016). In woody roots, rays and dilatation tissue (usually parenchyma cells) can represent lines of weakness (Cutler et al. 2009), from which fracture can propagate when root is subjected to a tensile stress. Moreover, environmental stresses such as waterlogging or drought can be experienced by perennial roots during particular years with consequent localized effects on tissues and biomechanical properties (Cutler et al. 2009; Loades et al. 2013). In particular, drought and waterlogging can affect wood structure. There is a strong correlation between soil water deficit and wood density, which can be attributed to a decrease in xylem vessel enlargement and the associated increase in the proportion of cell walls in wood tissue (Bouriaud et al. 2005). On the contrary, wet growing conditions result in the enlargement of the diameters of xylem vessels and the consequential decrease in wood strength (Arnold and Mauseth 1999; Alam et al. 2015). Consistently, both Striker et al. (2007) and Loades et al. (2013) found a decrease in root strength and Young's modulus in waterlogged plants (herbaceous species) due to aerenchyma development. Since the post-peak relationship between root strength and diameter generally accords with the literature, the negative power law model may be adopted with relative confidence for roots that have diameter larger than 2 mm (i.e., the common cut-off between fine and thin roots (Stokes et al. 2009). Applying this cut-off in the negative power law model

may hence avoid over-prediction of root strength provided in soil bioengineering applications.

Previous studies show that root function is not determined purely by root diameter (Pregitzer 2002). The physiology and life span of individual fine roots may vary in accordance with soil nutrients, degree of mycorrhizal infection and the position of a root on the branching root system (Pregitzer 2002). Some roots in a given diameter class can live longer and decompose more slowly than others, and hence, they are stronger for a longer period of time. King et al. (2002) reported that the presence of mycorrhizal fungi increases the life span of fine roots, while greater soil nitrogen concentration increases fine root development and mortality. This perspective may partially explain the observed differences of root biomechanics between our results and some reported data (Simon and Collison 2002; Bischetti et al. 2005; De Baets et al. 2008; Mickovski et al. 2009). Our results are for ten species on early stage of establishment, during which transplanting, optimal nutrients, water and soil density may have increased the growth and mortality of fine roots (Watson 1987; Pregitzer 2002). In contrast, many studies about woody roots have sampled root material from mature trees in natural forests (Bischetti et al. 2005; Bischetti et al. 2009; Vergani et al. 2014; Zhang et al. 2014).

Fine root life span, mortality and decomposition are the key factors that may affect the biomechanical properties of woody roots during plant establishment. Indeed, Watson et al. (1997) found that the mean tensile strength of *Kunzea ericoides* increased by 33% compared to living roots after 12 months since tree death, but subsequently the strength decreased to a value lower than that of live roots after 24 months. Root samples excavated from soil for biomechanical tests may include dead roots, which although are visually indistinguishable from live roots, have different biomechanical properties (Watson et al. 1997).

#### 4.4. Young's Modulus-diameter and Young's modulus-tensile strength relationships

The relationships between Young's modulus and diameter for the ten tested species generally showed similar trends to the corresponding strength-diameter relationships. Young's modulus and tensile strength were significantly correlated (Fig. 1 and Table 3). The  $R^2$  of the strength-modulus relationship for *B. sempervirens* and *E. europaeus* were 0.84 and 0.64 respectively. Moreover, those for, *C. monogyna*, *I. aquifolium*, and *P. spinosa* were higher than 0.40. Previous studies have shown that the relationships between Young's modulus and diameter

and those between tensile strength and diameter can have similar trends in both woody and non-woody roots (Fan and Su 2008; Mickovski et al. 2009; Loades et al. 2013). However, Young's modulus-diameter data are seldom reported in literature compared with the strength-diameter ones. Hence, the strength-Young's modulus correlations shown in Fig. 1 can help to fill this data gap.

Most existing root reinforcement models (i.e., (Wu et al. 1979; Waldron and Dakessian 1981; Pollen and Simon 2005) considered root tensile strength as the only biomechanical trait. Recently, Schwarz et al. (2010) highlighted the importance of Young's modulus when estimating mechanical reinforcement of vegetated slopes. Indeed, Young's modulus influence the mechanical activation of the root-soil interface interaction and hence the shear strength of root-permeated soil at different strain (Mickovski *et al.*, 2007). For a given amount of strain, stiffer roots mobilize more stress than softer roots and hence peak strength may be reached at smaller displacements during shearing. Young's modulus is a fundamental biomechanical trait that should be included in models of the progressive breakage of roots and the associated added shear strength to the parent soil.

#### 4.6. Root water status, shrinkage and tensile strength calculation

Root strength and Young's modulus are calculated on the basis of root cross-sectional area, which is very sensitive to uncertainty in diameter measurement (Eqns (2) and (3)). Root diameter depends strongly on root water status (Huck et al. 1970; Watson et al. 1997; Carminati et al. 2009; Carminati et al. 2013). In our experiment on the relationships between root water status and diameter for *C. scoparius*, root diameter showed a 30% diameter decrease as compare with the initial diameter (Fig. 3A). In particular, a large diameter decrease took place when the root water potential dropped below the permanent wilting point (-1.5MPa, Fig. 3B). If we assume a fully hydrated root with a diameter of 4 mm has a tensile capacity of 100 N, a root tensile strength of 8 MPa would be calculated. However, root shrinkages in diameter due to root water loss of 20 % and 30 % would lead to 1.5 times (i.e., 12 MPa) and 2 times (i.e., 16 MPa) greater root tensile strength, respectively, for a given applied force. This can be particularly relevant for young and fine roots with small tissues development, in which the water loss is generally faster than that in more developed and large roots (Taleisnik et al. 1999). A recent study reported by Yang et al. (2016) showed that root water content has a significant effect on root biomechanical properties. A small amount of water loss could increase root tensile strength, but oppositely, excessive water loss could



reduce root elasticity and root tensile strength. Therefore, we hypothesize that root water status may be one of the factors resulting in the large variation of tensile strength in fine roots as observed in literature, especially when roots were dried or rewetted before testing. Future work is needed to improve the understanding of the short- and long-term effects of root water status on root diameter, root biomechanical properties and the mechanical and hydrological reinforcements.

## 5. Conclusion

This study found contrasting patterns in root strength and Young's modulus data for the ten species during early stage establishment, potentially associated with different patterns of root development and growth. The following conclusions of this study can be drawn:

- The root tensile strength-diameter relationships of the ten species highlighted three different trends. The commonly-quoted negative power law in the literature was applicable for the strength-diameter data of only two out of the ten species tested, *E. europaeus* and *U. europaeus*. On the contrary, *B. sempervirens*, *I. aquifolium* and *P. spinosa* showed a slight increase in tensile strength with diameter.
- *C. avellana*, *C. monogyna* and *L. vulgare* consistently showed an initial increase in root tensile strength with diameter, reaching peak strength between 1.0 and 2.5 mm diameter. Beyond the peak strength, a reduction of strength is observed. These bimodal trends might be partially explained by the differences of the development stage of root primary and secondary structures.
- Indiscriminate use of the negative power law model to describe root tensile strength-diameter relation could overestimate root mechanical reinforcement, especially in the range of very small diameter roots where high root tensile strength would tend to be predicted by the model. Caution should be taken when this root biomechanical model is assumed for different taxa or for the same species that grows under different environmental conditions or during the challenging initial establishment period.
- Young's modulus was significantly correlated with tensile strength in all tested species. Root tensile strength may explain up to 80% of the variation in Young's modulus ( $R^2$ ) for individual species.
- Root diameter, investigated for Scotch Broom, depended strongly on root water potential and root water content by mass). Root water content could thus influence considerably the calculations of tensile strength..

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## Figures captions

**Fig. 1.** Morphological and biomechanical properties of tested species. From left to right are reported per each species i) Percentage of root length in each diameter classes between <0.1 and 5.0 mm (Means  $\pm$  standard error of mean; n= 5); ii) Root tensile strength (MPa) plotted against diameter (mm). Dashed lines represent the best-fit curve; iii) Root Young's modulus (MPa) plotted against diameter (mm); iv) Root Young's modulus plotted against tensile strength. Species acronyms: Bs (*Buxus sempervirens*); Ca (*Corylus avellana*); Cm (*Crataegus monogyna*); Cs (*Cytisus scoparius*); Ee (*Euonymus europaeus*); Ia (*Ilex aquifolium*); Lv (*Ligustrum vulgare*); Ps (*Prunus spinosa*); Sv (*Salix viminalis*) and Ue (*Ulex europaeus*).

**Fig. 2.** Boxplots of root tensile strength (A) and Young's modulus (B) per each tested species. Letters indicate significant differences among species, as tested using one-way ANOVA followed by post hoc Tukey's test (data were log transformed in statistical analysis). Species acronyms: Bs (*Buxus sempervirens*); Ca (*Corylus avellana*); Cm (*Crataegus monogyna*); Cs (*Cytisus scoparius*); Ee (*Euonymus europaeus*); Ia (*Ilex aquifolium*); Lv (*Ligustrum vulgare*); Ps (*Prunus spinosa*); Sv (*Salix viminalis*) and Ue (*Ulex europaeus*). The bottom and top of boxes represent the 25<sup>th</sup> and 75<sup>th</sup> percentile, while the line within the box marks the median. Whiskers (error bars) above and below the box indicate the 90<sup>th</sup> and 10<sup>th</sup> percentiles. Black circles are outlying points.

**Fig. 3.** (A) Relationship between root water content and root diameter for *C. scoparius* roots [Regression curve:  $f = 67.3 + 25.6 * x - 3.7 * x^2$ ; P-value < 0.0001;  $R^2 = 0.99$ ], and (B) root water potential and diameter relation for *C. scoparius* roots during progressive dehydration [Regression curve:  $f = 73.8 + 30.6 / (1 + \exp(-(x - 2.7) / -1.3))$ ; P-value < 0.0001;  $R^2 = 0.99$ ]. Mean values are reported  $\pm$  standard error of mean (n= 3 in Fig A; n= 8 in Fig B).



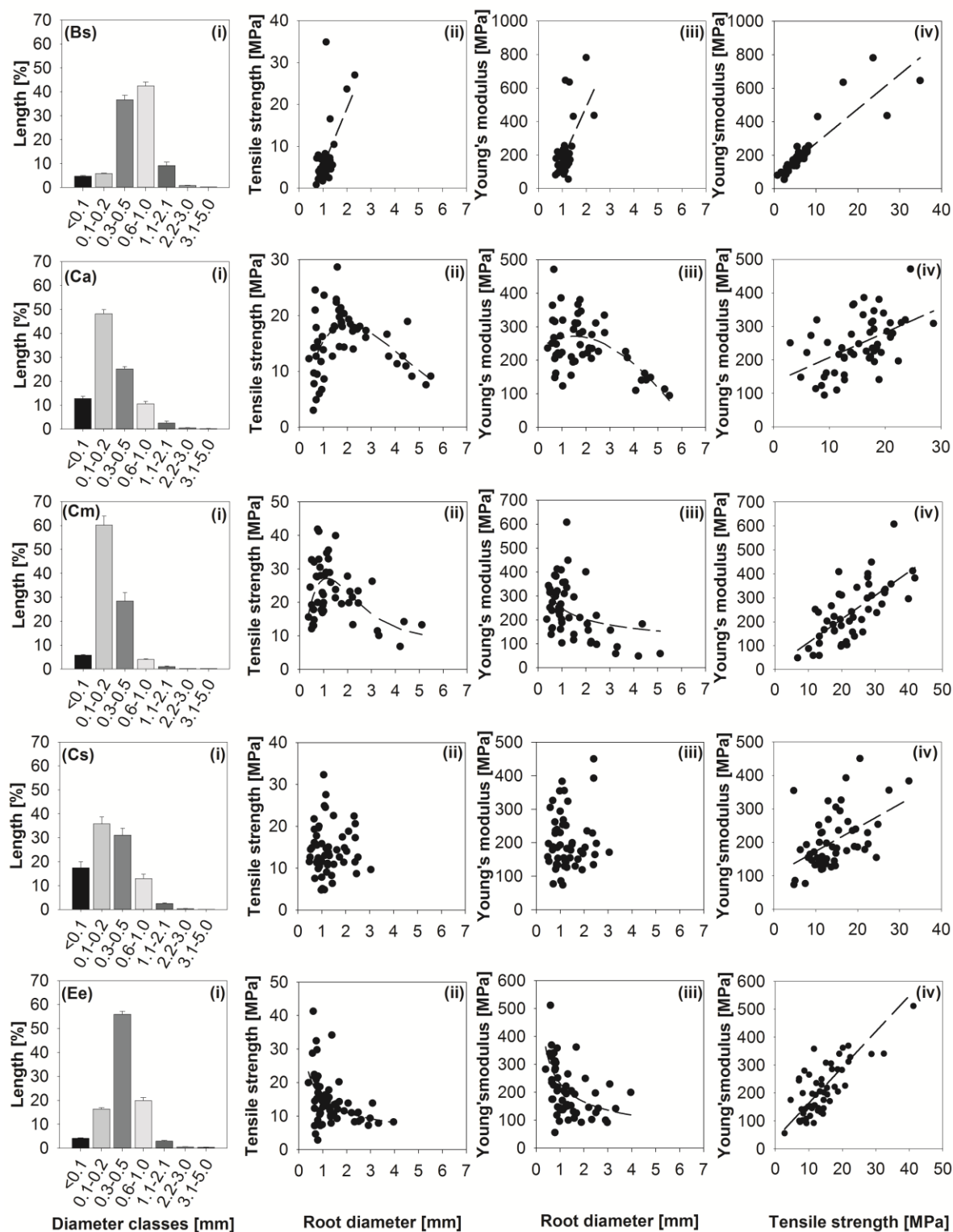


Figure 1 (page 1/2)



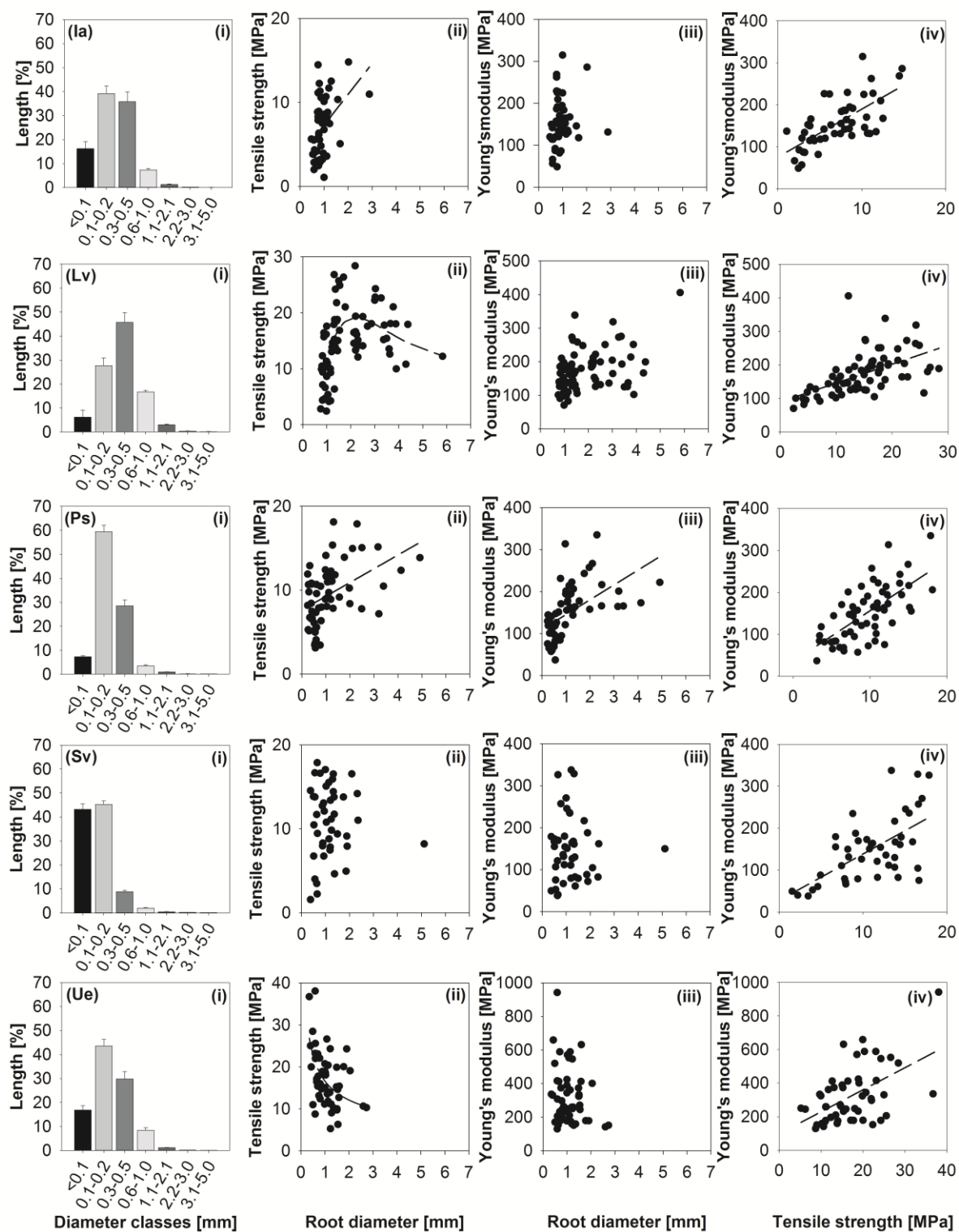


Figure 1 (Page 2/2)

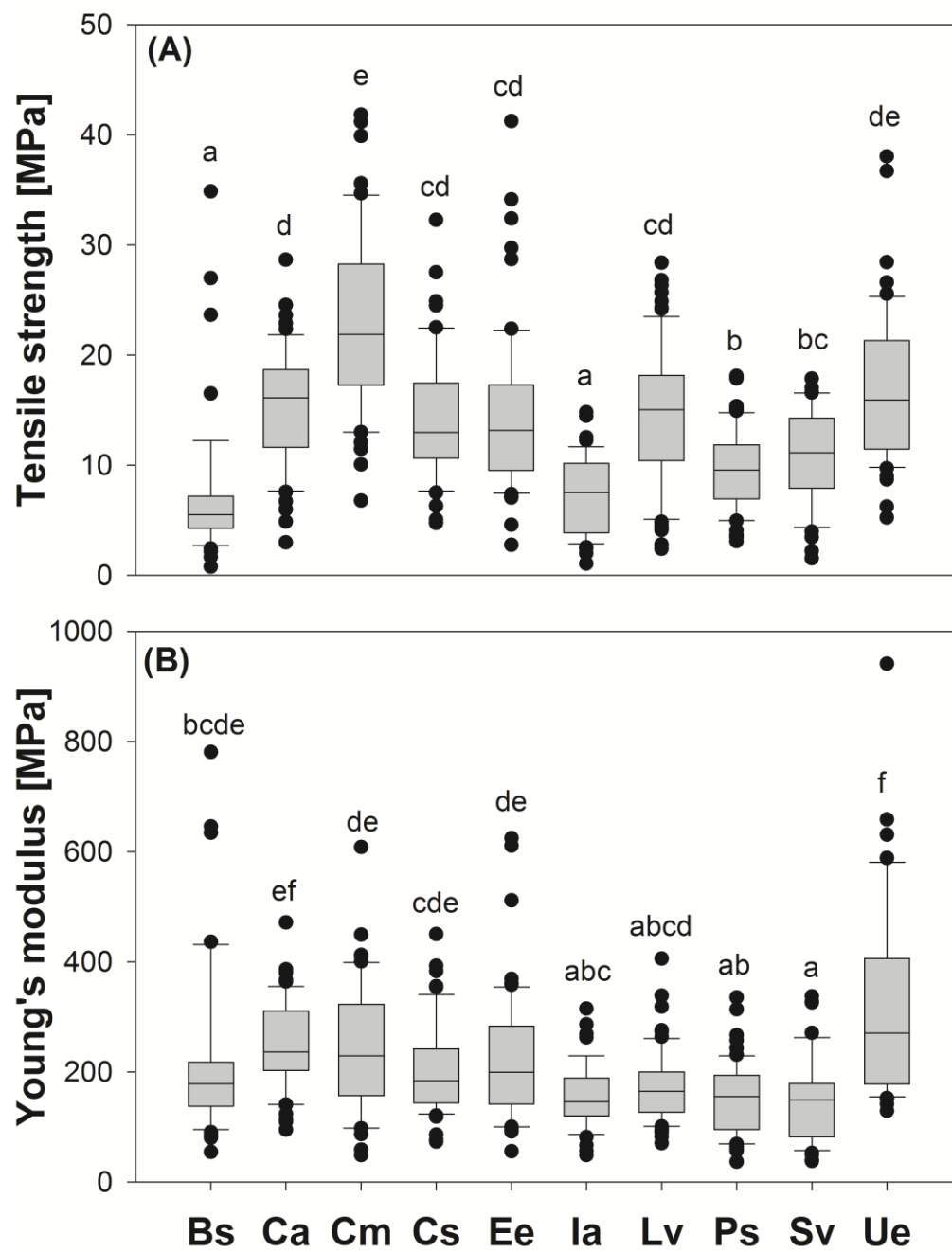
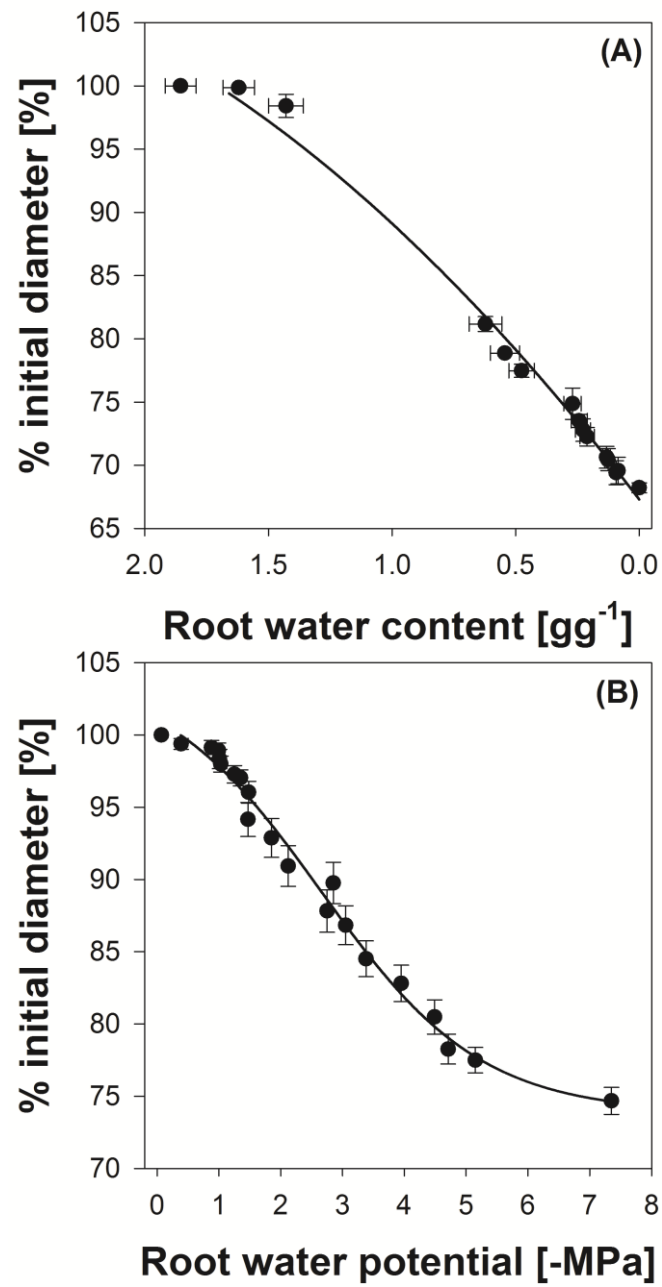


Figure 2



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740 Figure 3